



### Supplementary Figure S1. Morphogenesis of the paratympanic organ.

(a) Captured image from 3-dimensional reconstruction of the paratympanic organ (PTO) and surrounding tissues at stage 25 (E4.5) in chick. A local invagination of surface ectoderm (green) fuses with the pharyngeal endoderm (yellow).

(b) Transverse haematoxylin and eosin (H&E) stained section showing that internalised PTO ectoderm is associated with the tip of the pharyngeal pouch at stage 27 (E5).

(c) Serial H&E stained sections through the PTO region 12 hours earlier at stage 25 (E4.5) highlighting proximity of the first pharyngeal cleft (pc1), which remains open at this stage.

(d) Transverse section of PTO region at stage 29 (E6) immunostained for Islet1 (magenta) and activated caspase-3 (green) to highlight apoptotic cells. There are foci of apoptotic cells (green) ventromedial to the PTO (arrow).

(e) Magnified view of apoptotic cells (green) adjacent to the PTO (arrow).

(f) H&E staining of the same region reveals apoptotic bodies (arrow).

Scale bars: 100 μm. gg, geniculate ganglion; jv, jugular vein.